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The effect of maternal undernutrition on muscle development in the ovine fetus*

Berjan DEMİRTAŞ[1,**], Mukaddes ÖZCAN[2]

[1]Vocational School, Faculty of Veterinary Medicine, İstanbul University - TURKEY

[2]Department of Physiology, Faculty of Veterinary Medicine, İstanbul University - TURKEY

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Abstract: The effect of maternal undernutrition on muscle development of near-term ovine fetuses was studied in 2 experiments. In each experiment, Welsh mountain ewes were randomly assigned to either control (C) or nutrient-restricted (R) groups. In the first experiment, mild maternal undernutrition (85% of daily nutritional requirement (DNR)), and in the second experiment, severe maternal undernutrition (50% of DNR), were imposed between 0 and 70 days of gestation (dg). Controls for both groups were maintained on 100% of their DNR throughout the experiment. In each experiment, ewes were killed at 126 ± 1 dg by an intravenous injection of pentobarbitone. The semitendinosus muscle was dissected, and sections were stained for alkali-stable ATPase. The total number of primary and secondary fibers and the total number of fibers were estimated. In both experiments, there was a significant decrease in primary, secondary, and total fiber numbers ($P < 0.05$ for each). We speculate that the decrease in primary fiber number in both experiments is due to some of the large, central, and alkali-ATPase-negative (slow) fibers at near-term being secondary fibers. Therefore, the real primary fibers may not be affected.

Key words: Nutrition, gestation, muscle, fetus, sheep

Koyunda maternal besin kısıtlamasının fötus kas gelişimine etkisi

Özet: Koyunda gebeliğin ilk yarısında maternal besin kısıtlamasının gelişmiş fötusta kas gelişimi üzerine etkisi iki farklı deneyle yürütüldü. Her iki deneyde de koyunlar rastgele kontrol ve besin kısıtlaması yapılan gruplara ayrıldı. İlk deneyde hafif maternal besin kısıtlaması günlük besin ihtiyacı (GBİ)'nın % 85'i verilerek gebeliğin 0 ve 70. günleri arasında uygulandı. İkinci deneyde şiddetli maternal besin kısıtlaması (% 50 GBİ) gebeliğin 0 ve 70. günleri arasında uygulandı. Her iki deneyde kontrol grupları tüm gebelik süresince % 100 GBİ ile beslendi. Koyunlar her iki deneyde de gebeliğin 126 ± 1. gününde sakrifiye edildi. Fötuslar çıkarılarak semitendinossus kasları diseke edildi. Kesitler alınarak alkali-ATPaz ile boyandı. Kastaki primer, sekonder ve toplam kas lifi sayıları hesaplandı. Her iki deneyde de primer, sekonder ve toplam kas lifleri sayılarında belirgin bir azalma saptandı ($P < 0,05$). Primer liflerin sayılarındaki azalma koyunda merkezi, büyük ve alkali-ATPaz negatif (yavaş) liflerin bir kısmının morfolojik olarak primer lifler olarak gözükmesine rağmen aslında sekonder lifler olmalarından kaynaklanabilir. Bu durumda gerçek primer liflerin etkilenmediği söylenebilir.

Anahtar sözcükler: Besin, gebelik, kas, fötus, koyun

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** E-mail: berjan@istanbul.edu.tr

Introduction

Muscle fiber number is a major determinant of muscle mass. Muscle fiber hyperplasia occurs during the fetal period and is completed by birth in many agricultural animals such as sheep (1), cattle (2), and pigs (3,4). Fetal stage is crucial for skeletal muscle development because there is no net increase in muscle fiber number after birth (5). The number of fibers in adult muscle depends on the number of primary myofibers formed and the number of secondary myofibers that form around each primary myofiber. All primary fibers express initially slow myosin heavy chain (MHC) isoforms, and secondary fibers express fast MHC isoforms (6,7).

Muscle fiber number is affected by prenatal conditions in utero, including maternal nutrition (5,8,9). It seems likely that primary fibers are most indicative of genotype, whereas secondary fibers are more susceptible to many factors (10). Undernutrition of pregnant guinea pigs throughout gestation produces a reduction in fiber number by affecting the secondary fiber population; however, there appears to be no effect on the number of primary fibers in the biceps brachii muscles of the neonatal offspring (9).

In sheep, studies have been carried out on the effect of maternal nutrition on fetal and postnatal muscle fiber development (11-14) using different breeds, nutritional levels, and time periods. The first half of the gestation period is particularly critical; this is when myoblast proliferation occurs. Since skeletal muscle is particularly vulnerable to nutrient availability during fetal development (15), the level and the period of nutrient restriction is very important.

The aim of this study was to investigate the effect of mild and severe nutrient restriction, especially in early gestation, on fetal muscle development in Highland ewes.

Materials and methods

In both experiments, Welsh mountain ewes of uniform age, weight, and body condition score were mated with the same rams during the normal breeding season (November). In the first experiment, 13 ewes were randomly assigned to either the control group (C, n: 6) or the nutritionally restricted group (R, n: 7); in the second experiment, 14 ewes were randomly assigned to either the control group (C, n: 7) or the nutritionally restricted group (R, n: 7). In both experiments, the ewes were mated after estrus synchronization. The day of mating constituted the first day of gestation (dg). Progesterone levels in the blood samples were measured with ELISA at 16 dg. Ewes with low levels of progesterone were mated with the same rams again. Ewes were scanned with ultrasonography at 60 dg. Since singleton fetuses were used, twin-bearing ewes from experiment 1 (C, n: 1; R, n: 2) and experiment 2 (C, n: 2; R, n: 1) were excluded from the study.

Experiment 2 was performed to support the results obtained from experiment 1. Therefore, both experiments were conducted within a 1-year interval.

A hierarchical feeding system exists within groups of sheep; in order to permit specific regulation of individual nutritional intake, the animals used in this study were housed in individual pens. The floors of all pens were covered with wood shavings. Animals were allowed free access to water and were fed a complete pelleted diet. The diet consisted of barley, wheat, cooked cereal meal, micronized full-fat soya, grass meal, molasses, chopped straw, calcium carbonate, dicalcium phosphate, salt, and a sheep vitamin/mineral supplement. It provided 10.81 MJ/kg of metabolizable energy and 149.8 g/kg of crude protein, and it contained 88.4% dry matter. C animals were fed according to Agricultural and Food Research Council (AFRC) 1993 recommendations (16).

In experiment 1, R animals received 85% of their daily nutritional requirement (DNR) from the time of conception (0 dg) until 70 dg and then 100% of their DNR thereafter. In experiment 2, R animals received 50% of their DNR between 0 dg and 70 dg. Control animals were fed 100% of their DNR for the entire period of gestation.

Postmortem studies were carried out at 126 ± 1 dg in both experiments; animals were killed by intravenous injection of pentobarbitone. After slaughter, the fetuses were weighed and their crown-rump lengths (CRL) were measured. For all animals, the semitendinosus (ST) muscle was dissected and weighed, and a complete midbelly transverse

slice was rapidly frozen in liquid nitrogen. After preincubation at pH 10.4, sections of 10 μm were cut on a cryostat and stained for alkali-stable ATPase (17). For each animal, the total cross-sectional area of the muscle was determined. The numbers of primary and secondary fibers, based on ATPase staining and total fiber number, were counted in the frame areas. The frame areas transversed the muscle to take into account differences in the deep and superficial parts of the muscle; this was approximately 3% of the total cross-sectional muscle area. Large, central fibers that reacted negatively (stained less intensely) to ATPase at pH 10.4 were identified as primary fibers; intensely stained, peripheral fibers were classified as secondary fibers (1,12). These data were used to estimate the total number of fibers, the total number of primary fibers, and the number of secondary fibers. Kontron image analysis (KS300, Zeiss, Germany) was used for all measurements.

All procedures were carried out with local ethics approval of the Royal Veterinary College and in accordance with the regulations of the UK Home Office Animals (Scientific Procedures) Act, 1986.

Nutritional groups were compared using Windows SPSS 10.0 with the independent samples t-test. All values are presented as means ± standard error of mean (SEM). In all statistical tests, significance was accepted as $P < 0.05$.

Results

The results are obtained by images such as illustrated in Figure 1. Primary fiber number, secondary fiber number, and total myofiber number are shown in Figures 2 and 3.

In both experiments, there was a significant difference in primary, secondary, and total fiber numbers (Figures 2 and 3).

The decrease in total fiber number was 17.2% for experiment 1 (mild undernutrition) and 22.0% for experiment 2 (severe undernutrition). The decreases

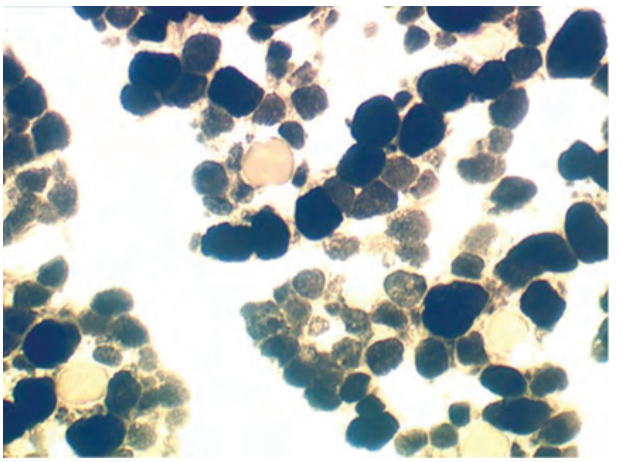

Figure 1. Myosin ATPase staining (pH 10.4) of fetal semitendinosus muscle from severely undernourished ewes at 126 ± 1 dg (40× objective).

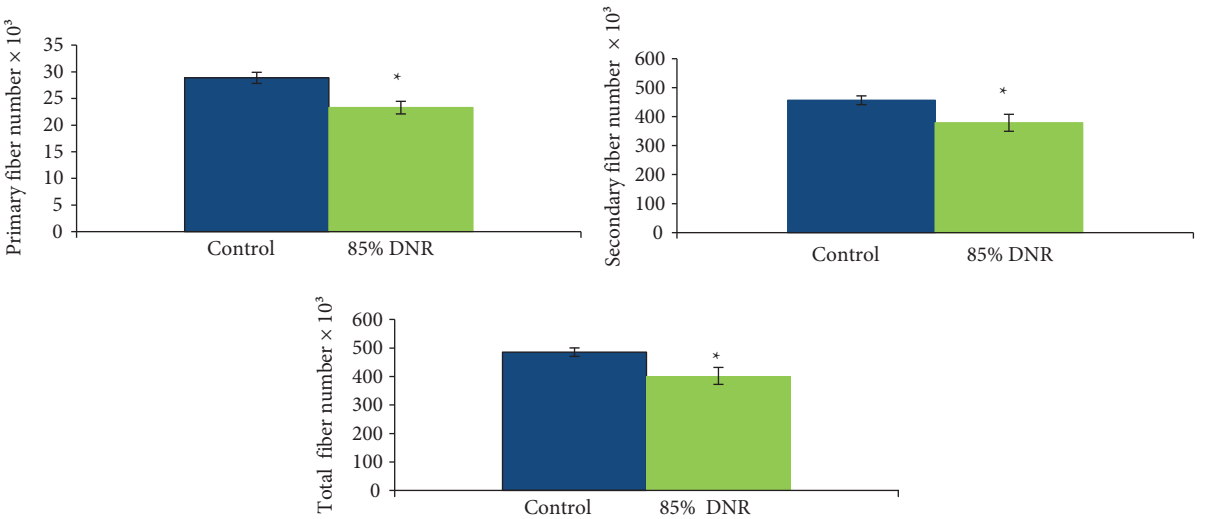

Figure 2. Numbers of primary fibers, secondary fibers, and total fibers in fetal semitendinosus muscles from control and mildly undernourished (85% DNR) ewes. For each parameter, the difference is significant ($P < 0.05$).

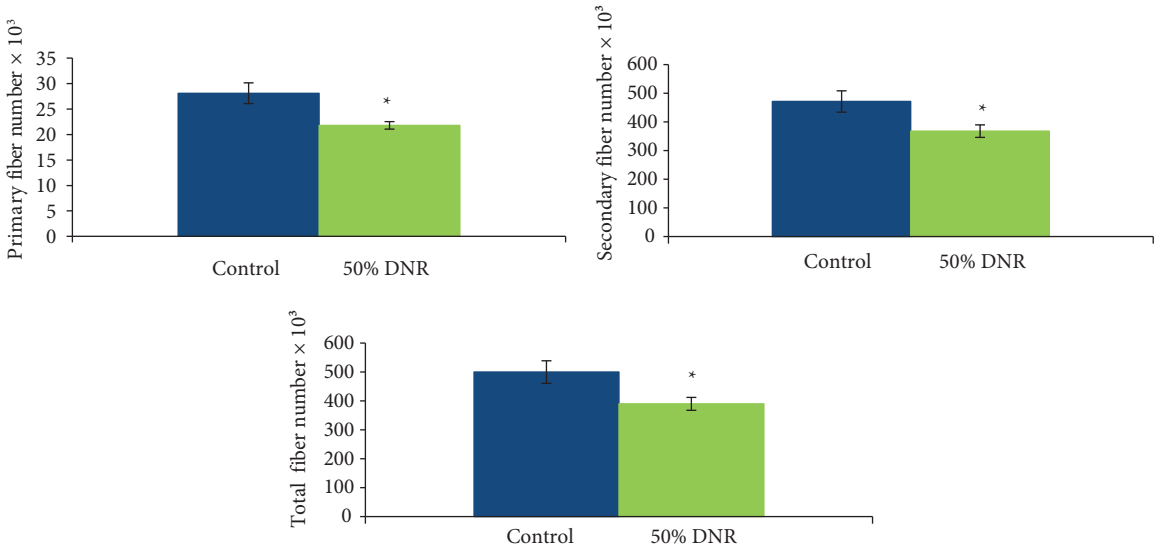

Figure 3. Numbers of primary fibers, secondary fibers, and total fibers in fetal semitendinosus muscles from control and severely undernourished (50% DNR) ewes. For each parameter, the difference is significant ($P < 0.05$).

in secondary fiber number and primary fiber number were 17% and 19.4% for experiment 1 and 22% and 22.4% for experiment 2, respectively (Table 1).

There were no significant differences between the groups for fetal weight or any other muscle measurements in both experiment 1 (Table 2) and experiment 2 (Table 3).

Discussion

There were no significant differences in fetal weight, CRL, or ST muscle measurements between mildly and severely undernourished ewes in our study. Gardner et al. (18) reported that there were no differences in the body weights of newborn lambs from early period undernourished ewes, whereas undernourishment in late gestation had a significant effect on fetal weight. The late gestation period has a greater effect on birth weight (19).

In this study, it was found that both mild and severe undernutrition in early pregnancy decreases total fiber number, secondary fiber number, and primary fiber number in near term fetuses.

Maternal constraint as a result of small placental size in autumn-lambing ewes resulted in significantly fewer muscle fibers in the ST muscle (20). A few studies showed that severe and moderate maternal undernutrition during different gestational periods caused a reduction in total fiber number in different sheep muscles. Zhu et al. (15) reported that 8-month-old lambs from severely undernourished ewes had fewer fibers in the longissimus dorsi muscle. Myofiber density in fetal triceps brachii muscles, but not in soleus muscles, was reduced with severely restricted maternal diets (14). The soleus muscle may

Table 1. Percentage decrease in total fiber, secondary fiber, and primary fiber numbers in restricted animals compared to control groups in experiment 1 and experiment 2.

	% decrease in total fiber number	% decrease in secondary fiber number	% decrease in primary fiber number
Experiment 1	17.2%	17%	19.4%
Experiment 2	22%	22%	22.4%

Table 2. Comparison of some parameters between control and 85% DNR groups in experiment 1.

Parameter	Control n: 5	85% DNR n: 5	Significance
Ewe weight (kg)	51.5 ± 2.35	47.6 ± 2.47	NS
Fetal weight (kg)	2.51 ± 0.23	2.84 ± 0.12	NS
Fetal CRL (cm)	47.7 ± 0.89	49.2 ± 1.35	NS
Fetal ST weight (g)	3.45 ± 0.43	4.08 ± 0.41	NS
Fetal ST length (cut out; mm)	51.3 ± 2.62	53.3 ± 3.12	NS
Fetal ST cross-sectional area (mm^2)	136.94 ± 12.23	14.88 ± 17.71	NS

All values are mean ± SEM.
NS: no significant difference in all parameters between control and 85% DNR ($P > 0.05$).
CRL: crown-rump length; ST: semitendinosus muscle.

Table 3. Comparison of some parameters between control and 50% DNR groups in experiment 2.

Parameter	Control	50% DNR	Significance
Ewe weight (kg)	50.7 ± 1.13	55.5 ± 3.15	NS
Fetal weight (kg)	2.30 ± 0.16	3.03 ± 0.44	NS
Fetal CRL (cm)	43.2 ± 1.15	46.5 ± 1.39	NS
Fetal ST weight (g)	4.05 ± 0.32	4.88 ± 0.48	NS
Fetal ST length (cut out; mm)	51.3 ± 4.67	56.6 ± 3.53	NS
Fetal ST cross-sectional area (mm^2)	142.16 ± 10.18	15.06 ± 10.58	NS

All values are mean ± SEM.
NS: no significant difference in all parameters between control and 50% DNR ($P > 0.05$).
CRL: crown-rump length; ST: semitendinosus muscle.

be less susceptible to prenatal undernutrition (9). Fahey et al. (13) reported that newborn lambs from severely undernourished ewes had fewer fibers in the vastus lateralis muscle but not in the ST. This may have resulted from the randomly chosen counting area and omission of the deep and superficial area. Using artificial insemination and the embryo transfer method, Quigley et al. (12) observed fewer fibers in the ST muscle in fetuses at 75 dg under maternal feed restriction during the periconception period.

In the current study of mildly and severely undernourished ewes, total muscle fiber numbers decreased significantly; however, there was no difference in ST muscle weights. There is evidence that muscle fiber number can change without any effect on muscle weight (12,21). Muscle weight is correlated with hypertrophy during the late fetal period. Maternal undernutrition during late gestation has an effect on fetal weight and muscle fiber diameter, but not number (5). In a late gestational nutrition challenge of singleton- and twin-bearing ewes, muscle mass was evaluated; muscle fiber diameter was limited, but not fiber number (22).

We found that both mild and severe undernutrition in early gestation caused a reduction in both primary and secondary fiber numbers. In other species, undernutrition in utero caused a reduction in secondary fiber number but not in primary fiber number (8). Although primary fibers are mostly genetically determined, there is evidence that primary fiber number may also be affected by administration of porcine somatotropin to pregnant sows between 10

and 24 dg (23). In both our experiments, the observed decrease in primary fiber number might be due to the very early restriction period, which encompassed conception and primary fiber formation. However, we speculate that the reduction in primary fiber number is most likely due to the large, central, ATPase-negative (slow) fibers originating from both primary and secondary generation fibers in sheep. Primary fibers form until 50 dg, whereas secondary fibers begin to form around 60 dg and up until 140 dg in sheep (1). In mammalian muscle, all primary fibers express initially slow MHC, and secondary fibers express fast MHC isoforms (6,7). However, Pin et al. (24) observed that secondary fibers can display different MHC expression. Furthermore, Maier et al. (25) found that large, central slow-expressing myofibers in sheep muscle increased up until 100 dg, and therefore originate from both primary and secondary fibers. Results of our ontogeny study in sheep muscle (unpublished data) were similar, showing that some secondary fibers had early expression of slow MHC (and stained negatively with alkaline-ATPase), migrated, and acted as a scaffold for the next generation of fibers. If this is the case, some of the large, central, slow MHC-expressing fibers (ATPase-negative) in near-term sheep muscle are in fact secondary fibers that appear to be primary.

The reported decrease in primary fibers is due to the fact that many of them are secondary fibers. In this case, secondary fibers alone are affected, and real primary fibers may be unaffected.

In conclusion, even mild undernutrition, especially in early gestation, affects the total muscle fiber number by reducing secondary fiber numbers, but probably not primary numbers, in ovine fetal muscle.

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